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# Drought-induced decline of productivity in the dominant grassland species *Lolium perenne* L. depends on soil type and prevailing climatic conditions

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ABSTRACT

Severe constraints on grasslands productivity, ecosystem functions, goods and services are expected to result from projected warming and drought scenarios under climate change. Negative effects on vegetation can be mediated via soil fertility and water holding capacity, though specific mechanisms are fairly complex to generalise. In field drought experiments, it can be difficult to disentangle a drought effect *per se* from potential confounding effects related to vegetation or soil type, both varying along with climate. Furthermore, there is the need to distinguish the long-term responses of vegetation and soil to gradual climate shift from responses to extreme and stochastic climatic events. Here we address these limitations by means of a factorial experiment using a single dominant grassland species (the perennial ryegrass *Lolium perenne* L.) grown as a phytometer on two soils types with contrasted physicochemical characteristics, placed at two elevation sites along a climatic gradient, and exposed to early or late-season drought during the plant growing season.

Warmer site conditions and reduced precipitation along the elevational gradient affected biogeochemistry and plant productivity more than the drought treatments alone, despite the similar magnitude in volumetric soil moisture reduction. Soil type, as defined here by its organic matter content (SOM), modulated the drought response in relation to local site climatic conditions and, through changes in microbial biomass and activity, determined the seasonal above and belowground productivity of *L. perenne*. More specifically, our combined uni- and multivariate analyses demonstrate that microbes in a loamy soil with low SOM are strongly responsive to change in climate, as indicated by a simultaneous increase in their C,N,P pools at high elevation with cooler temperatures and wetter soils. Contrastingly, microbes in a clay-loam soil with high SOM are mainly sensitive to temperature, as indicated by a strong increase in microbial biomass under warmer temperatures at low elevation and a concomitant increase in C:N, C:P and N:P ratios. High SOM promoted a better annual yield of the phytometer grass under warmer climate and the effect of drought on productivity was transient. In contrast, low SOM reduced cumulative yield under warmer conditions and root production strongly decreased, enduring a lasting drought effect. Microbes in soils with high organic matter remained more active during warmer and drier conditions, ensuring soil fertility and stimulating a higher overall plant nutrient availability and productivity.

Our study highlights the important role of soil type for grassland responses to both stochastic climatic extremes and long-term climate change. Management practices enhancing SOM accumulation via organic residue incorporation seem a promising way to mitigate the effects of increased temperature and drought on plants and soil microbes alike promoting thereby a sustainable ecosystem functioning.

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## 1. Introduction

Along with the pressure of climate change, grasslands are challenged to meet the growing demand for ecosystem services and for providing human goods (EEA, 2012). Under sufficient water and nutrient supply, grassland productivity could benefit from future climatic scenarios, i.e. higher $CO_2$ concentrations, higher temperatures and longer vegetation periods (Lüscher et al., 2005). However, sufficient water availability during the vegetation growing season might be at stake under certain future climatic predictions. For central Europe, for example, it is predicted that the occurrence of dry summers will increase by the year 2050 (IPCC, 2013) and that extreme drought events could become more frequent and intense in response to climate change (Ciais et al., 2005). In the western part of Switzerland, extreme summer droughts are predicted to cause 70% decrease in precipitations by the end of the 21st century (CH2011, 2011). Under such projected water deficits, we can then expect severe constraints on grassland productivity (Smith, 2011) as well as negative effects on other ecosystem functions, goods and services such as soil fertility, biodiversity and carbon (C) storage (De Vries et al., 2012; Jentsch et al., 2011).

The response of grasslands to drought and the degree to which ecosystem services are affected depend on the complex interactions among different factors involving plant community diversity, soil properties, climatic conditions and land management (see for example Deléglise et al., 2015; Thébault et al., 2014). Furthermore, duration, intensity and timing of the drought, as well as the frequency of rainfall events are all very important factors influencing the response of grassland communities to drought (Chou et al., 2008; Didiano et al., 2016). For example, using a mesocosm experiment, De Boeck et al. (2011) found that season of drought had a large effect on the magnitude of reduction in plant biomass and growth, with early summer drought having the largest impact followed by spring and then autumn droughts. This is likely because the early summer is a time of rapid plant development with leaf area at its peak and high temperatures favouring transpiration.

Higher growing-season temperatures and more frequent occurrence of drought periods have the capacity to alter C and nutrient cycling at ecosystem level, with a potential for significant plant–soil feedback (Melillo et al., 2002). Higher temperatures may lead to enhanced C losses from soils (Bond-Lamberty and Thomson, 2010) and faster nutrient cycling (Ineson et al., 1998), yet these increases may be offset, or even reversed, by reduced soil moisture (Davidson and Janssens, 2006). Drought stress and, more generally, lower soil moisture can reduce plant nutrient uptake by decreasing nutrient mineralization (Fierer and Schimel, 2002), nutrient diffusion and mass flow in soil (Chapin, 1991). When drought stress is followed by rewetting, this often results in enhanced mineralization (Canarini and Dijkstra, 2015), which has been attributed to nutrient release from dead microbial biomass (Borken and Matzner, 2009), or the decay of fine roots that have accumulated during the drying period (Sanaullah et al., 2012). Duration and intensity of drought, as well as frequency of drought-rewetting cycles will thus determine the net effect of drought stress on nutrient supply and soil organic C storage (He and Dijkstra, 2014). Therefore, while the ecophysiological response of plants to drought stress is rapid, soil fertility might respond differently and in a more complex way because of potential buffering capacities (Fridley et al., 2011).

Elevation gradients within a confined geographical region can be conceived as a natural climatic experiment where ecosystem processes can be primarily attributed to variation in temperature and precipitation. Soil monolith transplantation from high to low elevations has been shown to effectively simulate climate change (see for example Link et al., 2003; Mills et al., 2014), including a combined effect of temperature and precipitation. Alternatively, rain shelter experiments in grasslands have been increasingly used to single out drought effects. For example, Gilgen and Buchmann (2009) applied rain shelters to three grasslands at different elevations in Switzerland showing that there is not a general drought response, due to the different grassland types and associated plant functional types, local climate conditions and soil types. Such "space-for-time" substitutions make it difficult to disentangle the drought effect *per se* from potential confounding effects related to vegetation or soil type, which also vary along with climate. Another difficulty is disentangling long-term climate shifts (interpreted from elevation gradient analysis) from short-term climatic extremes (interpreted from drought treatments).

In an attempt to control for these confounding effects, we performed a pot experiment using two types of soils with different soil organic matter content, i.e. a loamy low-organic and a clay-loam high-organic soil, which were placed at two sites with different climatic conditions, and were further subjected to the effects of early and late-season droughts during the plant growing season. Perennial ryegrass (*Lolium perenne* L.) was grown in the pots as a phytometer since it is one of the most important forage grasses in Europe, rendering high yields and nutritional value. Moreover, because it has no distinct tolerance to drought, this forage species is likely to be particularly affected by climate change (Westermeier et al., 2016). With respect to plant productivity, we hypothesized that: (i) late-season drought has a stronger negative impact than early season drought, but that local climatic conditions interact with the timing in determining the direction and magnitude of the drought effect; (ii) long-term warmer and drier conditions at low elevation have a stronger effect on plant growth than short-term droughts, due to a combined reduction in assimilation capacity and in soil microbial activity and bioavailability of nutrients. Furthermore, with respect to soil properties, we hypothesized that (iii) drought effects on plant productivity are tempered in organic matter rich soils due to a higher ability to retain soil moisture, and (iv) higher soil organic matter content warrants positive effects of warmer climate in increasing microbial C, N and P pools, rendering these soils less prone to drought stress.

## 2. Materials and methods

### 2.1. Experimental design

The pot experiment took place within an existing rainout shelter experiment, which was installed in two permanent grasslands (sites) of Swiss Jura mountains in 2015–2016. One site was located in Chéserex (N 46°24′, E 6°10′) at 540 m a.s.l., representing conditions of low elevation (thereafter named "low site"), whereas the second site was located in St-George (N 46°30′, E 6°15′) at 945 m a.s.l representing conditions of high elevation ("high site"). Mean annual temperatures for the low and high sites were 10.4 and 7.6 °C, respectively, and mean annual precipitation were 1050 and 1290 mm, respectively. In this region, grasslands are highly productive (9–12 Mg DM $ha^{-1}$ $yr^{-1}$) and are regularly grazed by cattle or mowed for haymaking. The dominant plant species are the same in both sites: *Lolium perenne* L., *Trifolium repens* L., *Poa trivialis* L., *Poa pratensis* L., *Dactylis glomerata* L., *Phleum pratense* L. and *Taraxacum officinale* (L.) Weber ex F.H. Wigg.

Briefly, the rainout shelter experiment which was used for the pot experiment consisted in a complete randomized block design with five replicates (rain shelters of 12.0 × 6.4 m) set up in both sites on a grassland surface of approximatively 1 ha. Drought was simulated in plots of 4.5 $m^2$ (0.9 m × 5 m) within the rain shelters which included also the control plots. The periods of water stress were based on the seasonal dynamic of the growth (Fig. 1), either during the peak of growing season (treatment of early-season drought) or after the peak of growing season (treatment of late-season drought) for a period of 8 weeks. Based on the last 30-year precipitation average at each site, during a period of two months (see Table 1) control plots received 100% of precipitation whereas drought plots received 30% of precipitation simulating the periods of water stress (i.e. early-season and late-season drought). Before and after the period of water stress, drought plots received similar precipitation to the control plots (C).

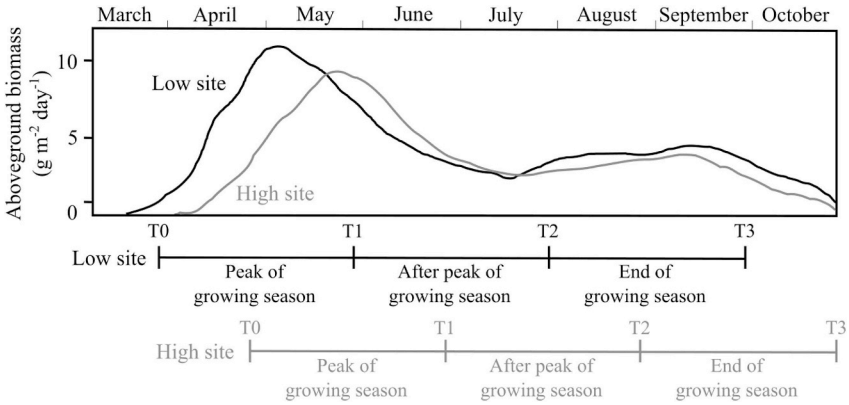


**Fig. 1.** Scheme of the seasonal dynamic of grassland biomass production for the two sites (unpublished data available from Agroscope institute). The different periods of plant growth along the season (each lasting for 8 weeks) are represented below the graph (Peak of growing season, After peak of growing season and End of growing season) with their respective sampling times (T1, T2 and T3). The 'peak of growing season' period has been centred on the peak of the vegetation growth curve and determined the beginning of the experiment at each site. T0 corresponds to the sampling time for initial conditions. The growth curve, periods and sampling times are drawn in black for the low site (540 m a.s.l.) and grey for the high site (945 m a.s.l.). Early-season drought was applied during the peak of growing season, while late-season drought was applied after the peak of growing season.

Watering was done twice a week by means of a flow controller and a custom-made watering stick, which was moved along the plots, thus mimicking a regular rain. The climatic conditions measured by a meteorological station installed at each site during the three periods of 8 weeks are given in Table 1. Mean vapor pressure deficit (VPD) was calculated for these periods as well. VPD is a measure of the drying power of air, therefore it directly influences the plant hydraulic capacity and, in turn, may impact rates of growth and overall plant health. VPD is calculated based on air relative humidity and air temperature as the difference between saturation and actual vapor pressure of air for a given temperature. A VPD threshold of 1.5 kPa was chosen to represent the approximate value above which stomata close under extremely dry air for most plant species (Sangines et al., 2018). This experimental design would allow disentangling long-term climate shift (site effect), from short time climate extreme (drought treatment effect).

The use of pots allowed to integrate in the experimental design the effect of soil type in 2016. Soils have been collected in close proximity to each shelter and selected so as to be representative of two contrasted features (e.g. fertility, SOM) of the same dominant soil at these altitudes, which is a cambisol (IUSS Working Group WRB, 2006). Soils had similar grassland species. At both sites we took the top soil (0–30 cm). The low site had a low-organic matter content (thereafter named "loamy low-organic" or "LOM"), and the high site had a higher organic matter content (thereafter named "clay-loam high-organic" or "HOM"). Besides the difference in organic matter content, these two soils differ also in their particle size fractions (determined on air-dried soils by sieving and the hydrometer method after removal of organic fraction with 30% $H_2O_2$ – Gee and Bauder, 1986), in their fertility and pH, being the HOM soil characterized by a finer texture and higher values of inorganic N, available P and pH as compared to the LOM soil (Table 2 and Supplementary table S1). Available water for plants in the soil (%) is comparable between both soils and it is calculated as difference in water content at pF = 4.2 and pF = 2.5 measured by means of a pressure plate (N = 10). Water content ranged between 34% and 33% at pF = 2.5 (field capacity), and between 18% and 19% at pF = 4.2 (permanent wilting point) for LOM and HOM soil, respectively.

In January 2016, both soils were collected up to a depth of 30 cm, larger stones and coarse organic residues removed, then used to fill pots of 9.3 L volume (diameter 20 cm, height 24.3 cm), which were stored under a roof at ambient winter temperature. In parallel, in a glasshouse under optimal growth conditions, shallow (3 cm) turfs of same diameter as the pots were prepared using each of the soils and sawn with the phytometer *Lolium perenne* L. (variety Salamandra 4n, Schweizer and Steffen, Switzerland) at a density of 0.7 g of seeds per pot. Following germination, the *Lolium* turfs were placed on top of pots, fertilised with a solution of N 27.5% (as ammonium nitrate), corresponding to 30 kg N $ha^{-1}$, and kept cool prior to their transfer to the field. This initial fertilization simulates a field common practice at the time of sowing (or after plant emergence). Another reason to apply this fertilizer was to get rapidly a dense plant cover and to standardize the initial plant available nutrient content in the two soils so as to avoid confounding effects at the start of the experiment. Altogether, the experimental design consisted in two sites (low *vs* high site), three drought treatments (plots: control, early-season and late-season droughts), two soil types (pots: loamy low-organic-LOM vs clay-loam high organic-HOM) and five replicates (rain shelters). In total there were 60 pots.

By end of March, once the morning frost events ceased at each site, the pots were transferred to the rain shelters. The timing of the entire procedure was calculated so as to have a delay of about 2–3 weeks between the placement in the low and high sites, and to match the vegetation seasonality at these two sites (Fig. 1). In each recipient plot, two pits were prepared and randomly received a pot with each soil type. Pots received the same watering regime as the entire plot.

### 2.2. Analytical methods

#### 2.2.1. Aboveground and belowground plant biomass

The standing crop of *Lolium perenne* was cut in all pots with scissors at time T1 (i.e., at the end of the early-season drought), T2 (i.e., at the end of the late-season drought) and T3 (i.e., at the end of the growing season period) (see Fig. 1). Plant material was dried at 65 °C to constant weight. At final harvest (time T3 – see Fig. 1), pots were extracted from the field plots and the surface (0–12 cm) and deep (12–22 cm) soil layers were separated. Soil samples were gently sieved (2 mm mesh) in the field to separate roots, which were washed in the laboratory before being dried at 65 °C and weighed. Soil was stored in a cooled box for temporary storage at 4 °C until further analyses, which started the day after the sampling.

#### 2.2.2. Soil respiration (Rs), soil temperature and moisture

All measurements were done four times, at the beginning of the drought treatment, when vegetation started to grow (T0), and immediately after the harvest of plant biomass, i.e., at the end of the early-season (T1) and late-season (T2) drought periods, and at the end of the growing season (T3) (see Fig. 1). On the day of measurements, all pots were shaded beforehand so as to avoid increase of soil temperature during the measurement session, which took place early in the morning after sunrise (between 8 a.m. and 11 a.m.). A customized funnel lid was tightly put on the pot and the measurement chamber was fixed on top of this lid. We used an infrared gas analyser (LiCor Li-8100) connected to a 10-cm survey chamber (LiCor 8100-102). Each measurement lasted 3 min, with a dead band of 30 s. Respiration fluxes were calculated from the increase in $CO_2$ concentration over time using an exponential regression and correcting for atmospheric pressure and temperature, as well as for the total volume under the chamber in each pot and the surface of the pot. Soil moisture, here corresponding to the water volumetric content (FieldScout TDR 300) and temperature were recorded in each pot, down to 12 cm, immediately after the measurement.

**Table 1**
Climate characteristics at the low and high elevation sites during the different periods of the experiment in 2016 and average 30-year rainfall, measured by a meteorological station installed at each site or nearby.

| Site | Periods (8 weeks) | Average rainfall (1981–2010) (mm) | Mean air temperature (°C) | Mean air humidity (%) | Mean vapor pressure deficit (VPD) (kPa) | Number of hours with mean VPD > 1.5 kPa |
|---|---|---|---|---|---|---|
| Low site | Early-season drought | 143 | 10.5 | 66.3 | 0.48 | 28 |
| 540 m a.s.l | Late-season drought | 154 | 18.0 | 69.4 | 0.78 | 215 |
| | End of growing season | 144 | 20.0 | 63.2 | 0.99 | 315 |
| High site | Early-season drought | 234 | 9.9 | 80.0 | 0.27 | 3 |
| 945 m a.s.l | Late-season drought | 213 | 16.7 | 72.1 | 0.59 | 48 |
| | End of growing season | 235 | 15.6 | 72.9 | 0.57 | 68 |

Precipitation applies for the conditions outside the rain shelters.

#### 2.2.3. Soil properties

Soil samples were analysed at the final harvest (T3 – see Fig. 1) for the top soil layer (0–12 cm) corresponding to the main rooting zone. The water content was determined gravimetrically by drying soil subsamples at 105 °C to a constant weight. Total carbon (C) and nitrogen (N) content in the bulk soil were analysed after ball milling. Soil C and N were determined under high-temperature oxidation using an elemental analyser (CE Instruments model NA2500 Nitrogen Carbon Analyser) and expressed as percent of dry soil weight. For the determination of microbial biomass carbon (Cmic) and nitrogen (Nmic), pairs of c. 5 g of fresh soil were weighed for each replicate and one sample from each pair was immediately extracted in 25 ml solution of 0.5 M $K_2SO_4$, whereas the other sample was put in a vacuum desiccator and subjected to chloroform vapor. After one day of fumigation, the fumigated soil sample was extracted with the same solution. Total C and N concentrations in the extracts of fumigated and non-fumigated samples were analysed by a TOC/TN analyser (Shimadzu TOC-V). To determine the soil available phosphorus (P) and the microbial phosphorus (Pmic), 3 g of fumigated and non-fumigated fresh soil was extracted with 40 ml of 0.5 M $NaHCO_3$ (Olsen method). Inorganic phosphorus content of the extracts was analysed by colorimetry using a spectrophotometer at 890 nm (Olsen et al., 1954). Microbial biomass C, N and P were estimated as the differences between the amounts of C, N and P after and before fumigation using an extractability factor of 0.45 for C (Vance et al., 1987), 0.54 for N (Brookes et al., 1985) and 0.4 for P (Brookes et al., 1982). Microbial biomass C, N and P and soil available P are expressed as $mg{\cdot}kg^{-1}$ oven dry soil. Ammonium ($N{-}NH_4$) and nitrate ($N{-}NO_3$) concentrations were determined by continuous flow analyses using an automated analyser (SEAL AA3 HR Autoanalyser) after extraction of 5 g of fresh soil with 30 ml of 1 M KCl and the results expressed as $mg{\cdot}kg^{-1}$ oven dry soil.

### 2.3. Statistical analysis

Statistical analyses were performed for all measured variables either at the different sampling times T1, T2 and T3 (volumetric soil moisture, soil temperature, above-ground biomass of *L. perenne*, soil respiration) or only at the final harvest T3 (total root biomass of *L. perenne* and root biomass at two depths, microbial biomass C, N and P, soil $NH_4$, $NO_3$, extractable P and C/N, C/P and N/P ratios). Overall ANOVA tests (three-way) and tests for each soil separately (two-way) are given in the figures and in Supplementary table S2. In the two-way ANOVA, two factors, i.e. site (S: experimental sites at low and high elevation) and watering regime (D: control, early-season and late-season drought), as well as their interaction were tested with block as a random factor. In the three-way ANOVA, soil was given as additional factor (G: LOM and HOM soil types), including interactions. Redundancy analysis (RDA) was done on the multivariate data from the final harvest (standardized) for each soil type, with drought treatment (categorical variable), soil temperature and soil moisture given as explanatory variables. Canonical axes and explanatory variables were tested subsequently with permutation tests. Assumptions of normality and homoscedasticity of the residuals in all final models were verified visually using diagnostic plots, with log transformations being applied to data when necessary. All analyses were carried out using R 3.4.2 (R development core team, 2017).

## 3. Results

### 3.1. Soil temperature and moisture and air VPD

Considering the comparable range of plant water availability of the two soils and their moisture content at pF 2.5 and 4.2 (Table 1), it can be concluded that changes in measured soil volumetric water content (TDR) reflect well the plant available water. Over the growing season, both soil types placed at the high site retained on average, significantly

**Table 2**
Main physico-chemical characteristics of the soil (0–30 cm) used for the experiment.

| Soil type | Total C (%) | Total N (%) | $pH_{H2O}$ | Clay (%) | Silt (%) | Sand (%) | Inorganic N mg $kg^{-1}$ | Available P mg $kg^{-1}$ | Available water (%) |
|---|---|---|---|---|---|---|---|---|---|
| Loamy low-organic (LOM) | 2.25 | 0.24 | 5.8 | 20 | 41 | 39 | 16 | 9 | 15.9 |
| Clay-loam high-organic (HOM) | 5.45 | 0.48 | 7.5 | 36 | 42 | 22 | 27 | 51 | 14.2 |

more soil water compared to the low site (LOM soil: +9%, +39%, +61%, and HOM soil: +16%, +19% and +81%, at T1, T2 and T3, respectively; Supplementary figure S3). Both early-season and late-season droughts strongly and significantly decreased mean soil volumetric moisture at both sites (LOM soil: -30% and −60%, and HOM soil: -39% and −43% at T1 and T2, respectively). The overall wetter HOM than LOM soils (+7%) provided for their significantly ($P < 0.05$) higher moisture content at T2 during the second part of the growing season and buffered significantly the negative effect of the drought treatment (Soil × Drought, $P < 0.001$). With respect to temperature, during the early growing season there was no temperature difference between the two elevation sites for both soil types (on average 13.1 °C) (Supplementary figure S4). Later in the season, however, both soils became increasingly warmer ($P < 0.001$) at the low site compared to the high site (for both LOM and HOM soils: +5.5 °C, +10.7 °C during late-season drought (T2) and end of growing season (T3), respectively). In addition, late-season drought (T2) induced slightly warmer conditions in both soil types (+0.9 °C; $P < 0.05$). HOM soils were on average slightly warmer than LOM soils (+0.2 °C; $P < 0.05$) during the entire growing season.

The mean air temperature during the early-season drought period was +0.6 °C higher at the low site (Table 1), and during the late-season drought and the following two months it was +1.3 °C and +4.4 °C higher, respectively. However, the water stress for plants, as quantified by the number of hours with mean VPD > 1.5 kPa, increased at the low site during the late-season drought and the following two months.

### 3.2. Above and belowground dry matter yield

Yield of *Lolium perenne* was significantly different between the two soils at all sampling periods (overall +43% in HOM soils, $P < 0.001$), with a strong interaction with site at the early-season drought (Fig. 2). Yield did vary for the same soil type depending on site elevation and season. For both soil types, there was more yield at the low site at the end of the early-season drought period (Fig. 2-T1; $P < 0.001$), but the trend reversed later in the subsequent harvests as the production at the high site increased (Fig. 2-T3; $P < 0.05$ for LOM soil, $P < 0.1$ for HOM soil). Drought had a weaker effect than site. Yield slightly decreased in both soil types during the late-season drought (Fig. 2-T2, $P < 0.05$), but this effect carried over until the end of the growing season only in the LOM soil at the high site (Fig. 2-T3, interaction, $P < 0.05$). With respect to the cumulative yield from the three harvests, it was different for both sites only in the HOM soil, with more yield at the low site (although marginally significant, $P < 0.1$) and drought effect persisted only in the LOM soil ($P < 0.01$). Interestingly, in the LOM soil, the early-season drought had a positive effect on aboveground plant production (Fig. 2-T1; $P < 0.01$).

Root production was also different depending on soil type (Supplementary figure 5: +15% total root biomass in HOM soils, $P < 0.05$, with interaction with site). Total root biomass in the LOM soil was significantly lower at the low site ($P < 0.05$), while in the HOM soil there was no difference between the two sites. Drought effect was only weakly significant, with interaction ($P < 0.01$) showing an increase of roots in the LOM soil in the late-season drought at the high site (both at 0–12 and 12–22 cm depth – data not shown), while in the HOM soil this increase occurred only in the deeper soil layer (data not shown). In both soil types, only a minor fraction of total roots was found in the deeper layer (data not shown).

### 3.3. Soil respiration

Soil respiration was significantly different at all sampling periods depending on soil type ($P < 0.001$) and interactions with site were observed (Fig. 3). Soils respired more at the low site at the end of the early-season (Fig. 3-T1; +25%, $P < 0.05$ for HOM soil) and late-season (Fig. 3-T2; LOM: +101% and HOM: +57%, $P < 0.01$) drought periods, as well as at the end of the growing season (Fig. 3-T3; LOM and HOM: +140%, $P < 0.001$). Drought treatment effect on respiration was marked in HOM soils already during the early-season drought (Fig. 3-T1; −26%, $P < 0.01$), but late-season drought impacted negatively the soil respiration most significantly in the LOM soil (Fig. 3-T2; −11% and −25% in early and late season drought, respectively, $P < 0.05$). For the LOM soil a legacy effect was still visible at the end of the growing season (Fig. 3-T3; −16% and −6% in early and late season drought, respectively, $P < 0.1$).

### 3.4. Microbial biomass

Soil type significantly affected microbial biomass C and N ($P < 0.05$) and there were highly significant interactions with site for all microbial biomass nutrients (Fig. 4). In each soil type site effect was generally highly significant for microbial biomass C, N and P (Fig. 4; $P < 0.01$ with the only exception of microbial P in HOM soil). The microbial biomass C, N and P pools increased in the LOM soil when it was placed at the high site and in the HOM soil when it was placed at the low site. The drought effect was observed only for microbial biomass C in HOM soil (Fig. 4a; $P < 0.01$). The ratios of microbial C:N, C:P and N:P increased significantly in HOM soil when transplanted at the low site (Supplementary figure S6; $P < 0.01$), while there were no significant changes in the LOM soil.

### 3.5. Soil available ammonium, nitrate and phosphorus

Soil type had an effect on soil nitrate ($P < 0.05$) and available P ($P < 0.001$), with strong interaction with site (Supplementary figure S7). LOM soil type did not show any significant pattern with respect to ammonium, nitrate and phosphorus. In HOM soil, nitrate showed a decrease under drought ($P < 0.05$) and concentration of available P was significantly reduced at low site ($P < 0.001$), while ammonium was affected neither by site nor by drought.

### 3.6. Multivariate analysis

RDA scatter plots with LOM and HOM soils (Fig. 5) shows that soil moisture and soil temperature are opposed along the first canonical axis, while drought treatment lies along axis 2. The general pattern was that total above-ground biomass was correlated to soil moisture, while soil respiration was correlated to temperature. Nitrate lies along axis 2 and it is more related to drought treatments, likewise the ratio above-belowground biomass. Most interestingly, microbial biomass N and P, as well as to some extent microbial biomass C, were related to soil moisture in the LOM soil, while in the HOM soil they were related to temperature. In HOM soil, microbial C:N, C:P and N:P ratios were strongly correlated to temperature, but not in the LOM.

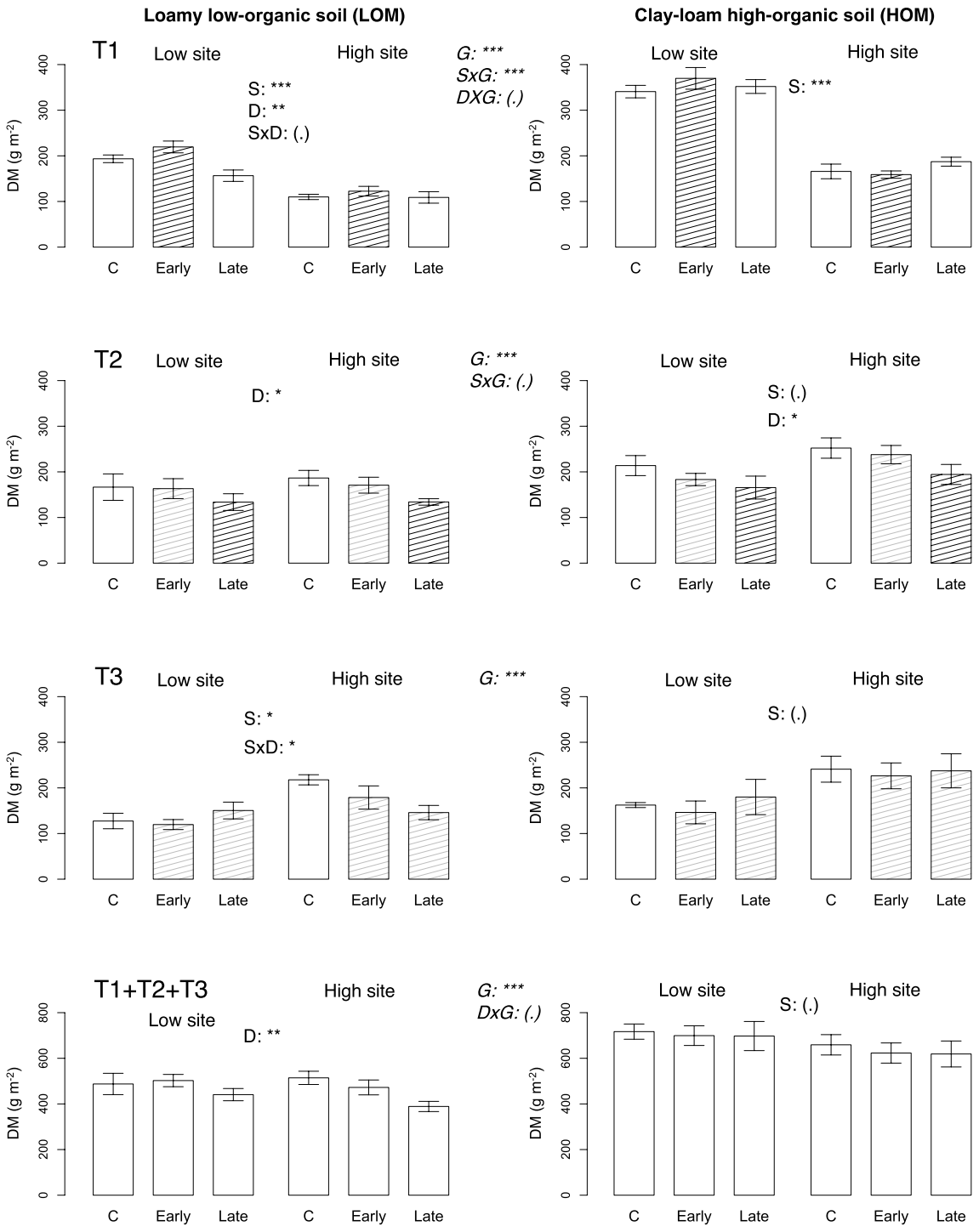


**Fig. 2.** Mean ( ± SE) above-ground biomass (N = 5) of *Lolium perenne* after the early-season drought (T1), the late-season drought (T2) and at the end of the growing season (T3) for the two soil types and the two sites. The cumulative above-ground biomass is given at the bottom (T1 + T2 + T3). Both soil types were transplanted each in the two sites at low (540 m a.s.l) and high (945 m a.s.l) elevation. Black hatching highlights when pots were experiencing drought, either early-season (Early, T1) or late-season drought (Late, T2) treatment, while grey hatching highlights pots that were previously subjected to drought. No hatching corresponds to either control pots (C) or to pots which did not yet undergo the drought treatment (e.g. Late in T1). ANOVA tests are given for each soil with site effect (S), drought effect (D) and their interaction (SxD), and the overall test is given in italics with soil effect (G) and interaction with S and D. Significance levels are $P < 0.001$ (***), $P < 0.01$ (**), $P < 0.05$ (*) and $P < 0.10$ (.).

## 4. Discussion

### 4.1. Seasonality of biomass productivity under different climatic conditions

In accordance with our first hypothesis, we found that local climatic conditions interact with the timing of drought occurrence to determine the direction and magnitude of shifts in plant productivity, based on the different stages of plant development. The higher mean air temperature at the low site during the early-season drought period favoured the growth of *L. perenne*, but later in the season the water stress for plants in relation to higher temperatures (e.g. VPD) increased drastically, causing a reduction in plant growth. Indeed, temperature has a

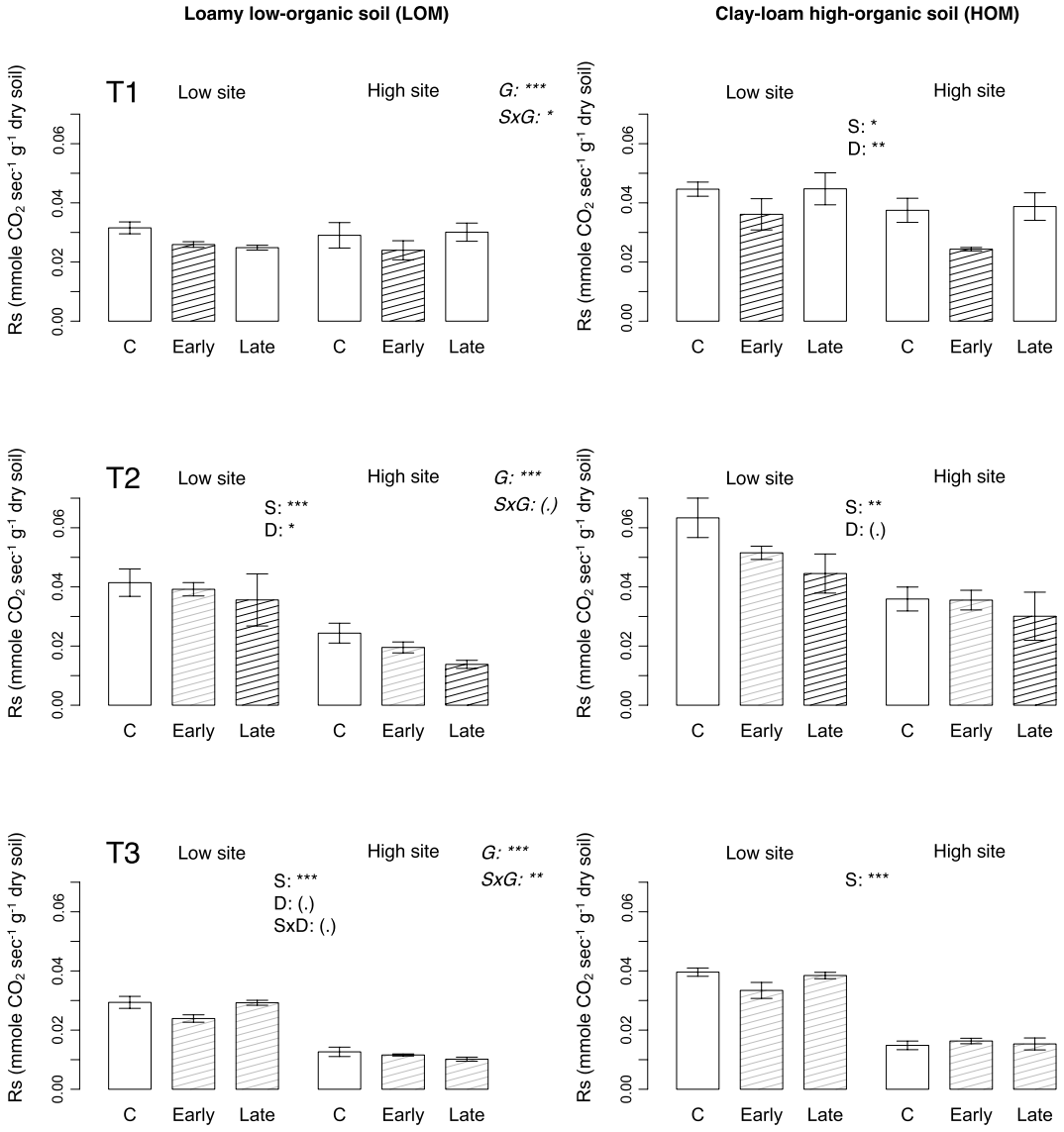


**Fig. 3.** Mean ( ± SE) soil respiration of the pots (N = 5) after the early-season drought (T1), after the late-season drought (T2) and at the end of the growing season (T3) for the two soil types and the two sites. See Fig. 2 for additional information.

beneficial role on the growth of temperate forage grasses, such as the perennial ryegrass *L. perenne* (Wingler and Hennessy, 2016). Nevertheless, the consequences of a prolonged water stress at landscape scale, as during the 2003 summer heat wave, were estimated in a 20–30% decrease of grassland production in Switzerland (Keller and Fuhrer, 2004). At plant species level, the most immediate effect of water stress is the limitation of plant growth through diminished leaf C fixation resulting from stomatal closure to prevent excessive transpiration (Signarbieux and Feller, 2012). Such growth reduction in response to experimental warming has also been observed in field-grown ryegrass (Nijs et al., 1996) and natural subalpine grassland communities (Gavazov et al., 2014; Mariotte et al., 2013). Furthermore, as hypothesized, late-season drought had a stronger negative impact than early-season drought, albeit this effect was inferior to the season-long combined temperature and precipitation shift along the elevational gradient, which determined the VPD for plant growth. It has been shown by Craine et al. (2012) that the inter-annual timing of climate variability is a factor determining the grassland productivity since, in addition to environmental conditions, developmental factors determine the growth of grasses (Hurtado-Uria et al., 2013). Noteworthy, the early-season drought seems to have promoted the growth of *L. perenne* in the LOM soil (Fig. 2-T1), which can be explained by the positive effect of drier soils on temperature conduction (Seneviratne et al., 2006) at the onset of spring growth. When considering the cumulative aboveground yield of *L. perenne*, the drought had a lasting effect only in the LOM soil. Conversely, in the HOM soil, *L. perenne* could retain the advantage of an earlier onset of the growing season with warmer climate at the low site, due to better ability of the soil to retain moisture that mitigates the negative effects of drought.

With respect to the below-ground biomass, late-season drought, which had a strong effect on soil moisture, promoted root growth at depth, especially within the LOM soil at the high elevation site. It seems that under unfavourable climatic conditions, *L. perenne* was able to invest relatively more resources belowground when deeper water resources were available. Such root plasticity has also been reported in multispecies communities (Hernandez and Picon-Cochard, 2016), in which vertical root complementarity was beneficial for water and nutrient uptake. Although we did not specifically measure fine root production, we could speculate that the observed depth increase of seasonal root biomass at the high site could have been related to a change in relative root size and therefore modified the potential uptake of nitrogen (Clarkson et al., 1986) and thus the recovery in plant biomass

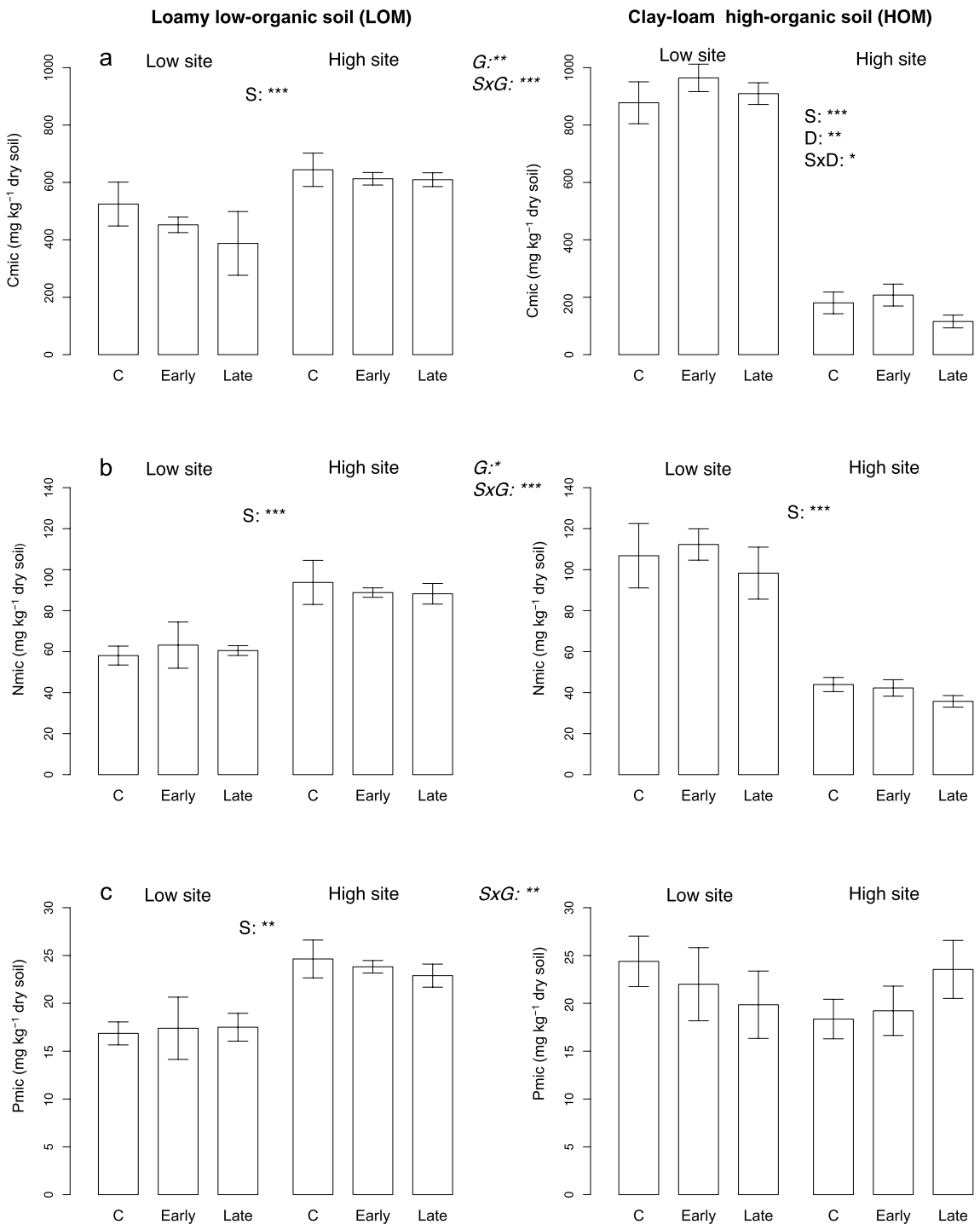


**Fig. 4.** Mean and SE (N = 5) soil (0–12 cm) microbial C (a), N (b) and P (c) at final harvest (T3) in the control pots and in the pots with early-season and late-season drought treatments for the two soil types and the two sites. See Fig. 2 for additional information.

production.

### 4.2. Effect on soil fertility and plant growth of long-term warmer and drier conditions vs short-term drought

In our experiment, the transplantation to a warmer and drier site had a stronger effect on soil fertility and plant growth as compared to the short-term drought treatments, despite a similar magnitude in soil moisture reduction. The soil microbial and chemical analyses were done only at the end of the growing season and therefore they do not strictly represent the response at the end of either of the two drought periods. However, they have the potential to highlight any drought legacy effect on microbial growth and nutrient mineralization. It seems that the legacy effect was absent, except for nitrate in the HOM soil, whose content was lower when soils had experienced a drought. In contrast, the warmer and drier soil conditions at the low site significantly reduced phosphorus availability and increased microbial C/P and N/P ratios in the HOM soil. Jupp and Newman (1987) found that the phosphorus uptake by *L. perenne* was reduced immediately after a period of drought due to reduced diffusion rate of phosphorus to the root surface in the drying soil, whereas after three weeks the recovery of uptake ability did occur. Although we could not evaluate the plant

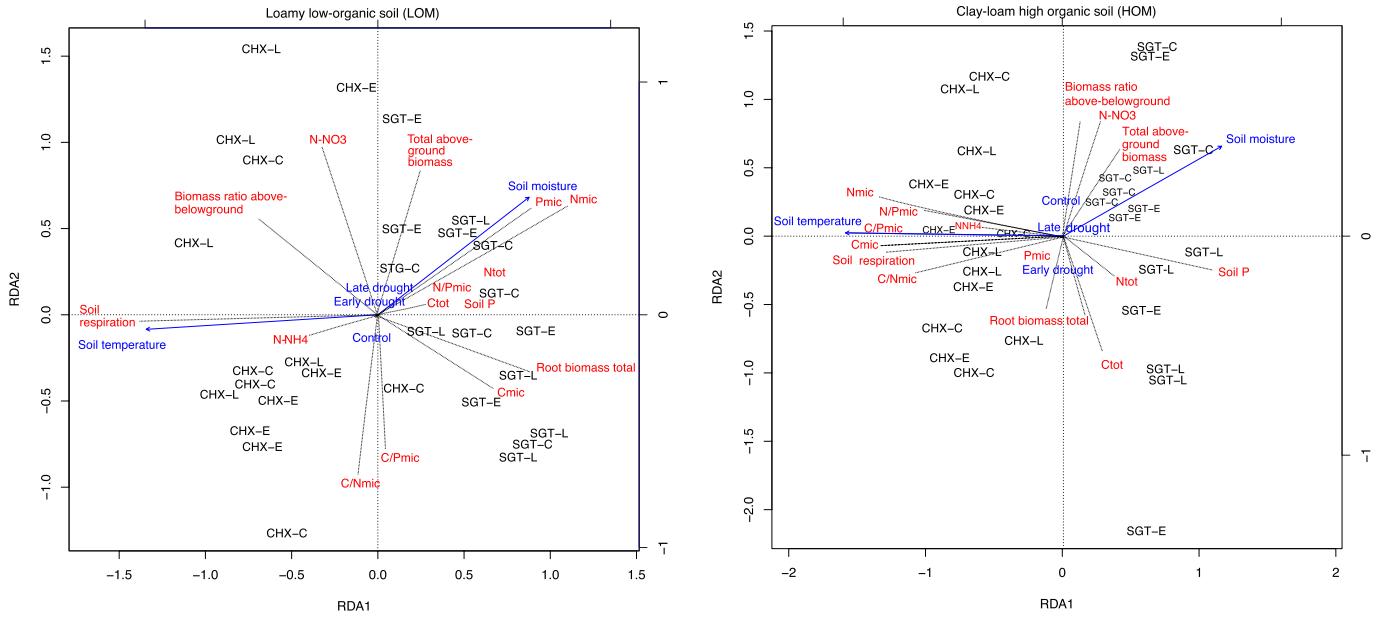


**Fig. 5.** Redundancy analysis (RDA) of the soil samples (surface 0–12 cm layer of the pot) for the loamy low-organic soil (graph on the left) and the clay-loam high-organic (graph on the right) placed at the low (540 m a.s.l) and high (945 m a.s.l) sites. Samples are labelled according to the recipient sites, CHX: Chéserex, SGT: St-George and to the treatments, C: control pots, E: early-season drought treatment, L: late-season drought treatment; thus, replicates have the same labels. Response variable are soil biochemical properties and biomass measures. Explanatory variables are treatments (control, early-season and late-season droughts), soil moisture and soil temperature. Left graph: axis 1: 21% ($P < 0.001$); axis 2: 7% (P = 0.10), overall model is significant ($P < 0.001$); soil temperature and soil moisture are significant ($P < 0.001$). Right graph: axis 1: 30% ($P < 0.001$); axis 2: 4.4% ($P = 0.44$), overall model is significant ($P < 0.001$); soil temperature and soil moisture are significant ($P < 0.001$).

uptake and possible limitation in the absence of chemical analyses of plant biomass, the decrease in productivity at the end of the growing season (Fig. 2-T3) for the warmer and drier HOM soils at the lower site seem to reflect this drop of extractable P. Indeed, in a meta-analysis, He and Dijkstra (2014) pointed to the increase in soil available N and a decrease in soil available P to explain the increase in plant N:P in response to drought.

Overall our findings suggest that long-term (growing season) climatic conditions and the warming-induced drying of air and soil affect soil biological activity and nutrient bioavailability more than stochastic drought events and confirms our second hypothesis. Considering that soil legacy effects have been demonstrated after repeated drought stress (Legay et al., 2018), it is plausible that we only detected biogeochemical changes in response to the long-term climate shift along the elevation gradient.

### *4.3. Opposed soil temperature and soil moisture limitation for different soil types*

Soils are diverse in mountainous regions, in particular for what concerns soil organic matter stocks and physicochemical characteristics, which makes predictions for their response to climate and land use change particularly difficult (Hagedorn et al., 2010). The two contrasting soil types responded differently to the two climate change treatments with respect to most of the measured physical, chemical and biological variables (Supplementary table S2). This was particularly noticeable for above-ground biomass and root biomass and it related also to the differences in fertility, in particular with respect to available N and P (Table 2). Microbial activity (here expressed as soil respiration and microbial biomass growth) differed significantly between the two soil types in response to a warmer and drier climate, likely due to inherent differences in the ability of the soil to retain moisture. Given that the rate of organic matter turnover in mountain grasslands is strongly dependent upon soil moisture only when temperature is not limiting microbial activity (Mills et al., 2014), it is not surprising that soil respiration rates in both soil types responded positively to the warmer conditions at low elevation. The soil moisture reduction induced by the drought treatment affected the respiration primarily in the LOM soil, especially during the late-season drought when this soil remained drier than HOM. Furthermore, in the LOM soil, the drought treatment had a legacy effect on soil respiration at the end of growing period (T3), two months after the late-season drought ceased, which indicates that soil microbial pool was affected beyond the drought period (Legay et al., 2018). The higher ability of HOM soil to retain moisture under field conditions is likely related to the combined effect of higher organic matter and clay contents, which together influence the soil structure. We presume that the soil structure of the HOM soil is such that it allows a better retention of precipitation water due to a lower rate of evaporation under drought, which could be explained by its pore's configuration. Overall, high SOM provides a buffered habitat for microbes (McGonicle and Turner, 2017; Xu et al., 2018), which in accordance with our third hypothesis can further mitigate drought effects on plant productivity due to a higher ability of the soil to retain moisture.

Interestingly, no direct drought effect was observed on microbial C, N and P pools (except for C in HOM soil) at final harvest, but both soil types showed a strong site elevation effect, with opposite directions with respect to soil temperature and moisture. A similar limitation trade-off between soil temperature and moisture has been previously demonstrated for soil respiration by Mills et al. (2014) who showed that, for the same type of soil transplanted along an elevation gradient, moisture was limiting at the warm and dry extreme, while it was temperature at the cold and moist extreme. These contrasting patterns are best illustrated in the RDAs, which showed that the LOM soil was prone to moisture limitation and thus could take advantage of the increased soil moisture at the higher site for increasing microbial biomass. Differently, the HOM soil, which was richer in soil organic matter and could sustain higher moisture, benefited from warmer soil conditions at the lower site in increasing microbial biomass. The microbial biomass C:N, C:P and N:P stoichiometry changed drastically only in the HOM soil, and their increase due to warmer climate was indicative of nutrient limitation for the increased microbial biomass. Warmer conditions did not affect soil extractable N for plant growth and only

slightly reduced P-availability in HOM, which in accordance with the fourth hypothesis, indicates that higher soil organic matter content warrants positive effects of warmer and drier climate on microbial activity and growth, rendering these soils less prone to drought stress.

## 5. Conclusion

In conclusion, our study shows that soils with different physico-chemical characteristics respond differently to drought in relation to site climatic conditions and that this impacts on biogeochemistry and plant productivity. Overall, soil with high organic matter content was less affected by drought than soil with low organic matter content. This indicates that soil type and management practices, such as SOM accumulation and organic residue incorporation (e.g. solid manure and/or slurry in pastures, or compost in cropland), represent a promising way to both curtail greenhouse gas emissions and mitigate the effect of increased temperature and drought on plant growth.

## Authors' contribution

AB conceived the ideas, designed methodology and drafted the paper, AB, TG, SP, GM, JQ collected the data, AB, PM, MM, TG, CS, LB and KG contributed to the interpretation of results and writing. All authors commented critically on the final version and gave final approval for publication.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2019.01.026.

## References

Bond-Lamberty, B., Thomson, A., 2010. Temperature-associated increases in the global soil respiration record. Nature 464, 579–582.
Borken, W., Matzner, E., 2009. Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils. Global Change Biology 15, 808–824.
Brookes, P.C., Powlson, D.S., Jenkinson, D.S., 1982. Measurement of microbial biomass phosphorus in soil. Soil Biology and Biochemistry 14, 319–329.
Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D.S., 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biology and Biochemistry 17, 837–842.
Canarini, A., Dijkstra, F., 2015. Dry-rewetting cycles regulate wheat carbon rhizodeposition, stabilization and nitrogen cycling. Soil Biology and Biochemistry 81, 195–203. https://dx.doi.org/10.1016/j.soilbio.2014.11.014.
CH2011, 2011. Swiss Climate Change Scenarios CH2011. 978-3-033-03065-7 Published by C2SM, MeteoSwiss, ETH, NCCR Climate and OcCC, Zurich, Switzerland, 88pp.
Chapin III., F.S., 1991. Effects of multiple environmental stresses on nutrient availability and use. In: Mooney, H.A., Winner, W.E., Pell, E.J. (Eds.), Response of Plants to Multiple Stresses. Academic Press, San Diego, CA, USA, pp. 67–88.
Chou, W.W., Silver, W.L., Jackson, R.D., Thompson, A.W., Allen-Diaz, B., 2008. The sensitivity of annual grassland carbon cycling to the quantity and timing of rainfall. Global Change Biology 14 (6), 1382–1394. https://dx.doi.org/10.1111/j.1365-2486.2008.01572.x.
Ciais, P., Reichstein, M., Viovy, N., Granier, A., Ogée, J., Allard, V., Aubinet, M., Buchmann, N., Bernhofer, Chr, Carrara, A., Chevallier, F., De Noblet, N., Friend, A.D., Friedlingstein, P., Grünwald, T., Heinesch, B., Keronen, P., Knohl, A., Krinner, G., Loustau, D., Manca, G., Matteucci, G., Miglietta, F., Ourcival, J.M., Papale, D., Pilegaard, K., Rambal, S., Seufert, G., Soussana, J.F., Sanz, M.J., Schulze, E.D., Vesala, T., Valentini, R., 2005. Europe-wide reduction in primary productivity caused by the heat and drought in 2003. Nature 437, 529. https://dx.doi.org/10.1038/nature03972.
Clarkson, D.T., Hopper, M.J., Jones, L.H.P., 1986. The effect of root temperature on the uptake of nitrogen and the relative size of the root system in Lolium perenne. I) Solutions containing both $NH_4^+$ and $NO_3^-$. Plant, Cell and Environment 9, 535–545.
Craine, J.M., Nippert, J.B., Elmore, A.J., Skibbe, A.M., Hutchinson, S.L., Brunsell, N.A., 2012. Timing of climate variability and grassland productivity. Proceedings of the National Academy of Sciences of the United States of America 109, 3401–3405.
Davidson, E.A., Janssens, I.A., 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440, 165–173.
De Boeck, H.J., Dreesen, F.E., Janssens, I.A., Nijs, I., 2011. Whole-system responses of experimental plant communities to climate extremes imposed in different seasons. New Phytologist 189, 806–817. https://dx.doi.org/10.1111/j.1469-8137.2010.03515.x.
De Vries, F.T., Liiri, M.E., Bjørnlund, L., Setälä, H.M., Christensen, S., Bardgett, R.D., 2012. Legacy effects of drought on plant growth and the soil food web. Oecologia 170, 821–833. https://dx.doi.org/10.1007/s00442-012-2331-y.
Deléglise, C., Meisser, M., Spiegelberger, T., Mosimann, E., Jeangros, B., Buttler, A., 2015. Drought-induced shifts in plants traits, yields and nutritive value under realistic grazing and mowing managements in a mountain grassland. Agriculture, Ecosystems & Environment 213, 94–104.
Didiano, T.J., Johnson, M.T.J., Duval, T.P., 2016. Disentangling the effects of precipitation amount and frequency on the performance of 14 grassland species. PLoS One 11. https://dx.doi.org/10.1371/journal.pone.0162310.
EEA, 2012. Climate change, impacts and vulnerability in Europe 2012 - an indicator-based report. EEA Report 12 ISSN 1725-9177 Copenhagen. https://dx.doi.org/9210.2800/66071.
Fierer, N., Schimel, J.P., 2002. Effects of drying–rewetting frequency on soil carbon and nitrogen transformations. Soil Biology and Biochemistry 34, 777–787.
Fridley, J.D., Grime, J.P., Askew, A.P., Moser, B., Stevens, C.J., 2011. Soil heterogeneity buffers community response to climate change in species-rich grasslands. Global Change Biology 17, 2002–2011. https://dx.doi.org/10.1111/j.1365-2486.2010.02347.x.
Gavazov, K., Spiegelberger, T., Buttler, A., 2014. Transplantation of subalpine wood-pasture turfs along a natural climatic gradient reveals lower resistance of unwooded pastures to climate change compared to wooded ones. Oecologia 174, 1425–1435. https://dx.doi.org/10.1007/s00442-013-2839-9.
Gee, G.W., Bauder, J.W., 1986. Particle-size analysis. In: Klute, A. (Ed.), Methods of Soil Analysis, Part 1. Physical and Mineralogical Methods, Agronomy Monograph No. 9, second ed. American Society of Agronomy/Soil Science Society of America, Madison, WI, pp. 383–411.
Gilgen, A.K., Buchmann, N., 2009. Response of temperate grasslands at different altitudes to simulated summer drought differed but scaled with annual precipitation. Biogeosciences 6, 2525–2539.
Hagedorn, F., Mulder, J., Jandl, R., 2010. Mountain soils under a changing climate and land-use. Biogeochemistry 97, 1–5.
He, M., Dijkstra, F.A., 2014. Drought effect on plant nitrogen and phosphorus: a meta-analysis. New Phytologist 204, 924–931.
Hernandez, P., Picon-Cochard, C., 2016. Presence of *Trifolium repens* promotes complementarity of water use and N facilitation in diverse grass mixtures. Frontiers of Plant Science 7, 538. https://dx.doi.org/10.3389/fpls.2016.00538.
Hurtado-Uria, C., Hennessy, D., Shalloo, L., O'Connor, D., Delaby, L., 2013. Relationships between meteorological data and grass growth over time in the south of Ireland. Irish Geography 46, 175–201. https://dx.doi.org/10.1080/00750778.2013.865364.
Ineson, P., Taylor, K., Harrison, A.F., Poskitt, J., Benham, D.G., Tipping, E., Woof, C., 1998. Effects of climate change on nitrogen dynamics in upland soils. 1. A transplant approach. Global Change Biology 4, 143–152. https://dx.doi.org/10.1046/j.1365-2486.1998.00118.x.
IPCC, 2013. Climate Change 2013: the Physical Science Basis. Intergovernmental Panel on Climate Change (IPCC).
IUSS Working Group WRB, 2007. World Reference Base for Soil Resources 2006, First Update 2007. World Soil Resources Reports No. 103. FAO, Rome92-5-105511-4.
Jentsch, A., Kreyling, J., Elmer, M., Gellesch, E., Glaser, B., Grant, K., Hein, R., Lara, M., Mirzae, H., Nadler, S.E., Nagy, L., Otieno, D., Pritsch, K., Rascher, U., Schädler, M., Schloter, M., Singh, B.K., Stadler, J., Walter, J., Wellstein, C., Wöllecke, J., Beierkuhnlein, C., 2011. Climate extremes initiate ecosystem-regulating functions while maintaining productivity. Journal of Ecology 99, 689–702. https://dx.doi.org/10.1111/j.1365-2745.2011.01817.x.
Jupp, A.P., Newman, E.I., 1987. Phosphorus uptake from soil by *Lolium perenne* during and after severe drought. Journal of Applied Ecology 24, 979–990.
Keller, F., Fuhrer, J., 2004. Agriculture and the heatwave of summer 2003. Agrarforschung 11, 403–420.
Legay, N., Piton, G., Arnoldi, C., Bernard, L., Binet, M.N., Mouhamadou, B., Pommier, T., Lavorel, S., Foulquier, A., Clément, J.C., 2018. Soil legacy effects of climatic stress, management and plant functional composition on microbial communities influence the response of *Lolium perenne* to a new drought event. Plant and Soil 424, 233–254. https://dx.doi.org/10.1007/s11104-017-3403-x.
Link, S.O., Smith, J.L., Halvorson, J.J., Bolton, H., 2003. A reciprocal transplant experiment within a climatic gradient in a semiarid shrub-steppe ecosystem: effects on bunchgrass growth and reproduction, soil carbon, and soil nitrogen. Global Change Biology 9, 1097–1105.
Lüscher, A., Fuhrer, J., Newton, P.C.D., 2005. Global atmospheric change and its effect on managed grassland systems. In: McGilloway, D.A. (Ed.), Grassland - A Global Resource. Academic Publishers, Wageningen, Netherlands, pp. 251–264.
Mariotte, P., Vandenberghe, Ch, Kardol, P., Hagedorn, F., Buttler, A., 2013. Subordinate plant species enhance community resistance against drought in semi-natural

grasslands. Journal of Ecology 101, 763–773. https://dx.doi.org/10.1111/1365-2745.12064.
McGonigle, T.P., Turner, W.G., 2017. Grasslands and croplands have different microbial biomass carbon levels per unit of soil organic carbon. Agriculture 7, 57. https://dx.doi.org/10.3390/agriculture7070057.
Melillo, J.M., Steudler, P.A., Aber, J.D., Newkirk, K., Lux, H., Bowles, F.P., Catricala, C., Magill, A., Ahrens, T., Morrisseau, S., 2002. Soil warming and carbon-cycle feedbacks to the climate system. Science 298, 2173–2176. https://dx.doi.org/10.1126/science.1074153.
Mills, R., Gavazov, K., Spiegelberger, T., Johnson, D., Buttler, A., 2014. Diminished soil functions occur under simulated climate change in a sup-alpine pasture, but heterotrophic temperature sensitivity indicates microbial resilience. The Science of the Total Environment 473–474, 465–472.
Nijs, I., Teughels, H., Blum, H., Hendrey, G., Impens, I., 1996. Simulation of climate change with infrared heaters reduces the productivity of *Lolium perenne* L. in summer. Environmental and Experimental Botany 36, 271–280.
Olsen, S., Cole, C., Watanabe, F., Dean, L., 1954. Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate. USDA Circular Nr 939, US Gov. Print. Office, Washington, D.C.
Sanaullah, M., Chabbi, A., Rumpel, C., Kuzyakov, Y., 2012. Carbon allocation in grassland communities under drought stress followed by $^{14}C$ pulse labeling. Soil Biology and Biochemistry 55, 132–139.
Sanginés de Cárcer, P., Vitasse, Y., Peñuelas, J., Jassey, V.E.J., Buttler, A., Signarbieux, C., 2018. Vapor pressure deficit and extreme climatic variables limit tree growth. Global Change Biology 24, 1108–1122.
Seneviratne, S.I., Luthi, D., Litschi, M., Schar, C., 2006. Land-atmosphere coupling and climate change in Europe. Nature 443, 205–209.
Signarbieux, C., Feller, U., 2012. Effects of an extended drought period on physiological properties of grassland species in the field. Journal of Plant Research 126, 251–261.
Smith, M.D., 2011. The ecological role of climate extremes: current understanding and future prospects. Journal of Ecology 99, 651–655. https://dx.doi.org/10.1111/j.1365-2745.2011.01833.x.
Thébault, A., Mariotte, P., Lortie, C.J., MacDougall, A.S., 2014. Land management trumps the effects of climate change and elevated $CO_2$ on grassland functioning. Journal of Ecology 102, 896–904. https://dx.doi.org/10.1111/1365-2745.12236.
Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry 19, 703–707.
Westermeier, P., Wosnitza, A., Willner, E., Feuerstein, U., Luesink, W., Schulze, S., Schum, A., Hartmann, S., 2016. Variation in drought tolerance of perennial ryegrass (*Lolium perenne* L.). In: Roldán-Ruiz, I., Baert, J., Reheul, D. (Eds.), Breeding in a World of Scarcity. Springer, Cham, pp. 63–68. https://dx.doi.org/10.1007/978-3-319-28932-8_9.
Wingler, A., Hennessy, D., 2016. Limitation of grassland productivity by low temperature and seasonality of growth. Frontiers of Plant Science 7, 1130. https://dx.doi.org/10.3389/fpls.2016.01130.
Xu, S., Li, P., Sayer, E.J., Zhang, B., Wang, J., Quio, C., Peng, Z., Diao, L., Chi, Y., Liu, W., Liu, L., 2018. Initial soil organic matter content influences the storage and turnover of litter, root and soil carbon in grasslands. Ecosystems 21, 1377–1389. https://dx.doi.org/10.1007/s10021-018-0227-3.